REMARKS

Reexamination and further and favorable reconsideration of the subject application in light of the following remarks, pursuant to and consistent with 37 C.F.R. § 1.114, are respectfully requested.

The Office Action Summary correctly indicates that claims 1-12 are pending in the application.

Claims 1-12 have been rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Wesley et al., *The Plant Journal*, 2001, vol. 27, no. 6: pp 581-590 in view of Yukawa et al., *Plant Molecular Biology*, 2002, vol. 50, pp 713-723 and Applicants' specification. The rejection is respectfully traversed.

The Office has noted that Wesley teaches post translational silencing of plant genes using ihpRNA as small as 98 base pairs and that dsRNA of at least 21 nucleotides in length have been associated with plants having post translational gene silencing. OFFICE ACTION dated January 25, 2007 at 4.

The Office has acknowledged that Wesley does not teach using a Pol-III type 3 promoter. *Id.* The Office has contended that Yukawa et al. teaches using a 7SL type 3 pol-III promoter and Pol-III type 3 promoters in general in plant cells because they were allegedly recognized in the art as strong promoters for driving gene inhibition in plants. OFFICE ACTION dated January 25, 2007 at 4. The Office has noted that the present specification teaches that Pol-III promoters of U3, U6, and 7SL were known in the art. *Id.*

The Office contended that it would have been obvious to use the promoter described by Yukawa et al. in the methods of Wesley et al. based upon general knowledge that such promoters could be used in genetic engineering of gene expression. *Id.* Applicants respectfully submit that a careful reading of Yukawa et al. shows that Yukawa et al. did not

suggest the use of type 3 Pol II promoters as broadly as the Office has contended. Yukawa et al. actually only noted that the previously recognized type 1 and 2 Pol III promoters are transcribed in all kinds of tissue and the transcripts are very stable. See, Yukawa at Abstract and Introduction. Yukawa et al. explained this as motivation for experimenting with a new class of pol III promoters exemplified by the 7SL gene. However, the experiments by Yukawa et al. would have been too preliminary to draw the same conclusion with respect to type 3 Pol III promoters. Applicants have pointed out that the disclosure of Yukawa et al. is actually limited to demonstrating that plant 7SL RNA genes with inserted antisense or ribozyme sequences are transcribed in an *in vitro* transcription system in a homologous plant extract. *See* Yukawa et al. at abstract line 7, page 714, bridging columns 1-2, or page 719, 2nd column lines 19-20. Yukawa et al. does not provide any evidence regarding how well chimeric genes comprising a type 3 Pol III promoter will actually be expressed *in vivo* in plant tissues.

Applicants maintain that the prior art fails to establish a proper prima facie case of obviousness. To establish a prima facie case of obviousness, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. M.P.E.P. § 2143. In particular, Applicants maintain that Yukawa et al. does not provide any suggestion or motivation that would make it obvious for a person of ordinary skill to modify Wesley et al. to make the now claimed combination. At most, Yukawa et al. suggests that pol III promoters of the class comprising 7SL should be the subject of future experiments. Yukawa et al. at 722, column 1, lines 29-33.

Even if the broad general suggestion to conduct further experiments using pol III promoters of the type exemplified by the promoter of 7SL by Yukawa et al. could establish a

prima facie case of obviousness, the presently claimed invention would still not have been obvious, because this type of promoter was only one of a very large number of promoters that would have been available to a person of ordinary skill in the art. The demonstration of *in vitro* expression by Yukawa et al. did not present any reason to a person of ordinary skill in the art to choose to use a type 3 pol III promoter over any of the many conventional types of plant expression promoters to control expression of a construct as now claimed. By contrast, the present disclosure demonstrated that the now claimed invention comprised a substantial improvement over prior art methods such as disclosed by Wesley et al. and the teachings of Yukawa et al. would not have taught a person of ordinary skill in the art that such an improvement was possible.

The present inventors have disclosed and claimed methods and constructs that provide substantial improvements over the prior art that could not have been predicted from the limited disclosure of Yukawa et al. The Office does not appear to have given adequate consideration to the surprising benefits accorded by the present invention. The Office has stated that the present claims "are not drawn to any comparative increase in expression using Pol III promoters relative to Pol II promoters." OFFICE ACTION dated October 22, 2007. Applicants respectfully submit that the claims are drawn to methods and constructs that the inventors have demonstrated comprise a substantial unanticipated improvement over the prior art.

Submitted herewith is a Declaration under 35 U.S.C. § 1.132 of inventor Ming-Bo Wang. Dr. Wang testified that Examples 2, 3 and 5 of the specification disclosed results of experiments performed by him and/or under his supervision that demonstrated that for short hairpins (ranging from 21 to 94 basepairs, i.e. from 42 to 198 nucleotides), the PolIII type 3

promoter driven chimeric genes result in a more pronounced silencing of the expression of the target gene than similar PolII driven chimeric genes.

Example 2 disclosed a comparison of gene silencing efficacy for a 41bp hairpin RNA (comprising GUS sequences) transcribed from a construct either driven by a PolII promoter (CaMV35S; pLMW53 see Table 2 on page 23, paragraph [0087]) or by a type 3 PolIII promoter (AtU3; pLMW58). These two constructs were introduced into two different tobacco plant lines expressing a GUS gene. The results of the MUG assay, allowing to determine the expression level of the GUS gene, indicate that the introduction of the PolII driven chimeric construct (Table 3 page 24, paragraph [0091]; entries in the column under the heading "53") did not reduce the expression of the GUS gene (compare with entries in the column under the heading "untransformed"). Introduction of a similar chimeric construct under the control of a type 3 PolIII promoter did result in a significant reduction of the GUS expression (see lower MUG readings in the column under the heading "58").

Example 3 disclosed a comparison of gene silencing efficacy for a 41bp hairpin RNA (comprising GUS sequences) transcribed from a construct either driven by a PolII promoter (CaMV35S; pLMW56 see Table 4 on page 25, paragraph [0094]) or by a type 3 PolIII promoter (AtU3; pLMW52). These two constructs were introduced into Arabidopsis thaliana plant lines expressing a GUS gene. The results of the MUG assay, which allows one to determine the expression level of the GUS gene, indicate that the introduction of the PolII driven chimeric construct (Table 5 page 26, paragraph [0098]; entries in the column under the heading "56") in this experiment did reduce the expression of the GUS gene (compare with entries in the column under the heading "untransformed"). However, introduction of a comparable chimeric construct under the control of a type 3 PolIII promoter resulted in lower

MUG readings indicating a more efficient reduction of the GUS expression (see lower MUG readings in the column under the heading "52").

In Example 5, PolIII promoters were tested for their efficiency in driving transcription of and silencing by short hairpin RNA targeting endogenous genes. In table 6, page 28, paragraph [0104], the results are summarized for short hairpins targeting phytoene desaturase (PDS) gene expression, transcribed either from a chimeric gene under control of a Pol III promoter (U6 promoter) or a PolII (CaMV35S) promoter. Introduction of the chimeric construct with CaMV35S promoter resulted in seedlings with either no bleaching or bleached cotyledons only, indicative a weak silencing of the PDS gene only, while most of the seedlings containing the chimeric construct with the U6 promoter were totally bleached, indicating stronger silencing of the gene expression.

Dr. Wang noted that the attached Exhibit 1 is a peer-reviewed scientific publication co-authored by him (Wang et al., 2008, RNA Vol 14, pp 903-913) which reports this data and expands the analysis towards the further generation of progeny plants (T2 population). In particular, the difference in PDS silencing was even more clearly manifest: 18 of 21 plant lines comprising the Pol III promoter driven chimeric gene showed intermediate to strong silencing, whereas only 5 of the 19 T2 plant lines comprising the Pol II promoter driven chimeric gene showed a visible but weak PDS silencing (Exhibit 1, page 904 right column, middle paragraph and Figure 3 (Exhibit 1, page 907).

Example 6 of the specification further describes the construction of chimeric genes targeted towards reduction of expression of the ethylene insensitive gene EIN2 either under control a Pol IIII type 3 promoter (ATU3B; pLMW162 and pLMW163; Table 7, page 30, paragraph [0111]) or under control of a Pol II promoter (CaMV35S; pLMW157 and pLMW158; Table 7, page 30, paragraph [0111]). The Pol III promoter-driven construct gave

significant EIN2 silencing as indicated by more vigorous leaf and root growth on ACC medium in comparison with wild-type Arabidopsis (ler) or the Pol II promoter-driven plants. The corresponding Pol II promoter CamV35S construct gave no significant EIN2 silencing in this experiment, showing that the Pol III promoter construct was much more effective than the Pol II promoter construct for EIN2 gene silencing. Exhibit 1, page 907, top of right column describes that also for this endogenous gene, the chimeric construct directed by the Pol III type 3 promoter conferred more efficient silencing than the CaMV 35S driven construct.

From the examples disclosed in the specification, a person of ordinary skill in the art would conclude that the type 3 Pol III promoters are more effective than the CaMV35S promoter for gene silencing with relatively short hairpin sequences, such as hairpin sequences ranging from about 19 basepairs to about 200 bp. Dr. Wang testified that this discovery could not have been predicted from the state of the art at the time the invention was made, including the teaching of the references that have been cited by the Examiner.

Dr. Wang concluded that the combined teachings of the above mentioned references would not have allowed a skilled person to predict that type 3 Pol III promoters are more effective than the CaMV35S promoter for gene silencing with relatively short hairpin sequences. At the time of the invention, a large variety of promoters of various types would have been known to a person of ordinary skill in the art. However, the state of the art regarded the CaMV35S promoter as a strong constitutive promoter for directing expression in plants. Absent any specific evidence that another type of promoter would provide significantly better results than the CaMV35S, there would have been no motivation for a person of ordinary skill to use a different promoter. In particular, there would have been no reason for a person of ordinary skill in the art to select the type 3 polymerase III promoters

that are recited in the claims of the present application over any of the several other types of promoter for use instead of the CaMV35S promoter.

Thus, the specification disclosed the substantial unanticipated improvement afforded by the presently claimed invention. The Office may not disregard this evidence because an analysis of obviousness of a claimed combination must include consideration of the results achieved by that combination. *The Gillette Co. v. S.C. Johnson & Son Inc.,* 16 USPQ2d 1923, 1928 (Fed. Cir. 1990). An understanding of the particular results achieved by the new combination is critical to the analysis. *Id.* (citing *Interconnect Planning Corporation v. Feil,* 227 U.S.P.Q. 543, 551 (Fed. Cir 1985)). Applicants submit that an analysis which takes appropriate account of the substantial improvement that the inventors have accomplished with the claimed invention must conclude that the invention was not obvious. Absent any disclosure anticipating the significant improvements afforded by the present invention, there would have been no reason to modify the teachings of Wesley et al. to make the present invention. Thus, the surprising benefits afforded by the present invention do comprise a secondary consideration that demonstrates that the invention would not have been obvious.

For at least the foregoing reasons, reconsideration and withdrawal of the remaining rejection in this application is respectfully requested.

CONCLUSION

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned concerning such questions so that prosecution of this application may be expedited.

The Director is hereby authorized to charge any appropriate fees that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800.

Respectfully submitted,

BUCHANAN INGERSOLL & ROONEY PC

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